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Neurospora as part of an undergraduate genetics course.

T. Ishikawa

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Abstract

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The following contribution presents, in outline form, procedures for experiments with Neurospora that are used in a genetics course at the University of Tokyo. The nature of accompanying laboratory lectures is indicated for each experiment, as well. The students, about 15 per class, are undergraduate majors in biology who meet for 5 hours per week for 12 weeks; they are expected to come to the laboratory outside regular class hours to make observations whenever necessary. Media, cultures and sterile glassware for the experiments are prepared by the students, themselves. Several of these exercises may be run simultaneously, depending upon the class schedule.

Experiment 1. Characterization of mutant strains.

Lecture: Mutation, types of mutants, pathway of adenine (AMP) synthesis.

Strains: ad-3; ad-4; ad-6; ad-8; ad-8; lys-5; ylo-1; asco, lys-5; 74A; 3. la.

Procedure:

1) Prepare fresh cultures of mutants and the wild types given. Use glycerol complete slant medium + adenine 100 ug/ml and lysine 100 ug/ml.

2) Sterilize petri dishes, test tubes and pipettes. Prepare sterile water blanks.

3) Prepare media: a) sucrose agar plating medium with and without supplements; b) liquid minimal medium with and without supplements. Supplements: adenine 100 ug/ml, hypoxanthine 100 ug/ml, lysine 100 ug/ml or adenine + lysine.

4) Pour 20 ml agar medium, autoclaved, into each plate. Dispense 1 ml liquid medium into each 10-cm tube, plug autoclave.

5) Observe morphological characters of fresh slant cultures.

6) Make conidial suspensions of each strain in water and shake well.

7) Inoculate conidial suspensions into both media. Mark the strain numbers on plates and tubes and incubate at 25C.

8) Observe growth after 1-3 days. Record morphological and biochemical characters of the strains.

Experiment 2. Allelism test.

Lecture: Discussion of gene, locus, allelism; cis-trans test.

Strains: ad-8 mutants (E6A; E129A; E32A; E1l1A; E34A; E41A); ad-4A.

Procedure:

1) Prepare fresh cultures of mutant strains given. Use glycerol complete slant medium (+adenine 100 ug/ml).

2) Prepare plates of minimal agar medium and sterile water blanks. Sterilize pipettes.

3) Observe morphological characters of fresh slant cultures. Suspend conidia of each strain in 2 ml water.

4) Mark on the bottom of each minimal agar plate 4 spots on the periphery: one spot for two individual mutants and two spots for the combination of these two mutants. Make all possible mutant combinations.

5) Inoculate the spots marked on the bottoms of the plates with conidial suspensions of the appropriate mutants, using 1-ml pipettes. Incubate at 25C.

6) Observe the results every day for at least 3 days. Consider allelic relationships among the mutant strains given. Construct the complementation map for the allelic mutants.

Experiment 3. Recombination and the genetic map (1).

Lecture: Meiosis, tetrad analysis, recombination, genetic map.

Strains: asco, lys-5; 74A.

Procedure:

1) Prepare fresh cultures of both strains. Use glycerol complete slant medium (+ lysine 100 ug/ml).

2) Prepare slants of crossing medium (+ lysine 100 ug/ml) in 3 x 18-cm tubes.

3) Inoculate conidia of both strains into a slant tube. Incubate at 25C for 2 weeks.

4) Observe at least 100 asci under the microscope and calculate the centromere distance for the asco locus.

Experiment 4. Recombination and the genetic map (2).

Lecture: Linkage, linkage groups, genetic map.

Strains: ad-8, lys-5, ylo-1, a; 74A.

Procedure:

1) Prepare fresh cultures of the mutant and wild type given. Use glycerol complete slant medium (+ adenine 100 ug/ml and lysine 100 ug/ml). Prepare crossing medium, supplemented, in 18-cm tubes.

2) From fresh slant cultures, inoculate mutant and wild conidia onto crossing medium. Incubate at 25C for 3-6 weeks, until ascospores are shot.

3) Prepare plating medium (sorbose minimal agar medium + adenine + lysine), 0.05% agar solution and sterile plates.

4) Prepare ascospore suspension in 0.05% agar solution. Count the number of ascospores in 0.05 ml suspension. Heat shock the ascospores at 60C for 40 minutes in a water bath.

5) Pipette spore suspension (200 spores per plate) into sterile plates. Pour medium (40C) into plates containing ascospores. Mix well. Incubate at 25C for 3 days.

6) Prepare isolation medium (minimal medium + 0.5% agar + adenine + lysine). Melt, pour into 7-cm test tubes, plug and autoclave.

7) Isolate 200 colonies; cut out a piece of colony and transfer it into isolation medium. Incubate at 25C for 5-7 days.

8) Prepare test medium (minimal liquid medium with appropriate supplements poured into 7-cm tubes and sterilized).

9) Observe the characters of each isolate and test for biochemical requirements by inoculating conidia into liquid test medium suitably supplemented.

10) What is the genotype of each isolate? Consider the linkage relationships.

Experiment 5. <u>One gene-one enzyme relationship</u>.

Lecture: Protein synthesis; amylases produced by Neurospora.

Strains: 74A; amylase mutant which shows no amylase activity in the culture filtrate.

Procedure:

1) Prepare fresh cultures of amylase mutant and wild type. Use glycerol complete slant medium.

2) Prepare crossing medium in 18-cm tubes.

3) From fresh slants inoculate mutant and wild type into crossing medium. Incubate at 25C for 4 weeks.

4) Prepare plating medium (sorbose minimal medium).

5) Suspend ascospores in 0.05% agar solution. Count the number of ascospores in 0.05 ml suspension. Heat shock ascospores at 60C for 40 minutes.

6) Plate 200 ascospores per plate. Incubate at 25C for 3 days.

7) Prepare isolation medium (minimal medium in small tubes).

8) Isolate 100 colonies. Incubate at 25C for one week.

9) Prepare: a) minimal liquid medium + 1.5% maltose in 20 18-cm tubes (3 ml/tube), and b) minimal liquid medium + 0.2% starch + 1% sucrose in 100 10-cm tubes (1 ml/tube). Plug and autoclave.

10) Inoculate conidia of each isolate, amylase mutant and wild strain into a and b media. Into a put 20 isolates, and into b put 100 isolates. Procedure b is a convenient simple method to observe the presence of amylase activity in the culture filtrate. Procedure a is used to confirm the b result. Incubate at 25C; a for 2 weeks, b for 5 days.

11) After 5 days add to the b cultures 0.1 ml of I_2-KI solution. Identify amylase mutants.

12) After 2 weeks, pipette out 0.4 ml culture filtrate from the a cultures and assay for amylase activity. Identify the amylase mutants and compare with the result from procedure b.

Assay method:

Reaction mixture: to 1.6 ml of 0.11% starch dissolved in 0.05M tris buffer (pH 6.0) add 0.4 ml of culture filtrate. Incubate at 37C for 60 minutes. Stop reaction by adding 0.4 ml 1N HCl.

Starch-iodine reaction: to 1 ml of reaction mixture add 0.5 ml 1N HCl and 1 ml of I_2(0.03%)-KI (0.3%) solution. Dilute to 10 ml by adding 7.5 ml water.

Measure transmittance at 660 mu.

Experiment 6. Induction of mutation.

Lecture: Mutagenesis, forward and back mutations.

Strains: ad-8 (E6A).

Procedure:

1) Prepare a fresh culture of the ad-8 mutant strain. Use glycerol complete medium in 16 100-ml flasks supplemented with adenine, 100 ug/ml.

2) Prepare plating media: a) to detect revertants, minimal sorbose medium, 15 plates for each irradiation, b) for survival test, minimal sorbose medium + adenine 100 ug/ml, 15 plates for each irradiation.

3) Prepare a conidial suspension of the mutant in sterile water. Spin down the conidia in centrifuge tubes. Wash 3 times with water by centrifuging. Resuspend in 90 ml water. Estimated number of conidia should be approximately 1×10^8 ml.

4) Pipette 10 ml of conidial suspension into a sterile petri dish and irradiate with a 15W UV-lamp at 30 cm. for between 0 and 30 minutes, taking samples at 5 minute intervals.

5) Dilute irradiated suspension for survival test, using sterile dilution blanks (9 ml of water) and 1 ml pipettes. Make dilution of 10^{-3}-10^{-6} depending upon the killing rate estimated.

6) Pipette diluted suspensions into plates. Pour in agar medium at 40C and immediately mix well. Plate irradiated diluted suspension onto medium b, using 3 dilutions, 5 plates each. Plate irradiated suspension without dilution onto medium a; 10 plates, 0.8 ml inoculum and 5 plates, 0.1 ml inoculum. Incubate at 25C for 4 days.

7) Count the number of colonies per plate. Draw the survival curve and mutation frequency-UV dose curve.

Experiment 7. Cytoplasmic inheritance.

Lecture: Function of mitochondria, nature of poky mutant, results of reciprocal crosses.

Strains: poky A; poky a; 74A; 3. la.

Procedure:

1) Prepare fresh cultures of mutants and wild type. Use glycerol complete slants.

2) Prepare crossing medium in 18-cm tubes.

3) Inoculate protoperithecial parents: inoculate conidia of all four strains, separately, into tubes of crossing medium. Incubate at 25C for 7 days.

4) Make suspensions of conidial parents in sterile water (all four strains). Pour 0.8 ml of conidial suspension into a tube containing a protoperithecial parent culture. Make all possible reciprocal combinations. Incubate at 25C for 2 weeks.

5) Prepare ascospore suspensions in 0.05% agar solution. Count the number of ascospores in 0.05 ml suspension. Heat shock the ascospores at 60C for 40 minutes.

6) Plate the spore suspensions (500 ascospores per plate) on minimal medium + 1.5% sucrose + 1.5% agar.

7) Observe the size of the colonies after 4 days' incubation at 25C.

- - - Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.